

Research Note

Fecal Carriage of Extended-Spectrum β -Lactamase–Producing *Enterobacteriaceae* in Swine and Cattle at Slaughter in Switzerland

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MS 10-372; Received 5 September 2010/Accepted 10 November 2010

ABSTRACT

During the past decade, extended-spectrum β -lactamase (ESBL)–producing *Enterobacteriaceae* have become a matter of great concern in human medicine. ESBL-producing strains are found in the community, not just in hospital-associated patients, which raises a question about possible reservoirs. Recent studies describe the occurrence of ESBL-producing *Enterobacteriaceae* in meat, fish, and raw milk; therefore, the impact of food animals as reservoirs for and disseminators of such strains into the food production chain must be assessed. In this pilot study, fecal samples of 59 pigs and 64 cattle were investigated to determine the occurrence of ESBL-producing *Enterobacteriaceae* in farm animals at slaughter in Switzerland. Presumptive-positive colonies on Brilliance ESBL agar were subjected to identification and antibiotic susceptibility testing including the disc diffusion method and E-test ESBL strips. As many as 15.2% of the porcine and 17.1% of the bovine samples, predominantly from calves, yielded ESBL producers. Of the 21 isolated strains, 20 were *Escherichia coli*, and one was *Citrobacter youngae*. PCR analysis revealed that 18 strains including *C. youngae* produced CTX-M group 1 ESBLs, and three strains carried genes encoding for CTX-M group 9 enzymes. In addition, eight isolates were PCR positive for TEM β -lactamase, but no *bla*_{SHV} genes were detected. Pulsed-field gel electrophoresis showed a high genetic diversity within the strains. The relatively high rates of occurrence of ESBL-producing strains in food animals and the high genetic diversity among these strains indicate that there is an established reservoir of these organisms in farm animals. Further studies are necessary to assess future trends.

Antimicrobial resistance in bacteria has emerged as a problem in both human and veterinary medicine. Of current interest is a resistance mechanism in *Enterobacteriaceae* that reduces the efficacy even of modern expanded-spectrum cephalosporins and monobactams; the mechanism is based on plasmid-mediated production of enzymes that inactivate these compounds by hydrolyzing their β -lactam ring. These enzymes, called extended-spectrum β -lactamases (ESBLs), are variants of well-known Ambler type A serine β -lactamases. Around their active site, they have undergone one to three amino acid exchanges that enable them to extend their substrate spectrum to include oxymino cephalosporins and monobactams. While many ESBLs are derivatives of classical broad-spectrum β -lactamases such as TEM-1 or SHV-1, members of other families like CTX-M, Oxa, PER, and KPC have recently been reported more frequently (5). Moreover, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole.

Since the first description of ESBL-producing *Enterobacteriaceae* isolated from hospitalized humans (14), many

nosocomial outbreaks have been reported. However, for several years there has been an increase in the isolation of ESBL-producing strains in the community (18). More recently, various reports have discussed dissemination of ESBL-producing *Escherichia coli* in healthy food-producing animals in several countries in Europe and Asia (6, 8, 10, 19, 23) or in food products like meat, fish, and raw milk (11–13). Very recently Wittum et al. (24) and Doi et al. (7) described ESBL producers in healthy dairy cattle and retail meat in the United States.

Therefore, the impact of healthy farm animals as a reservoir for ESBL-producing *Enterobacteriaceae* in the food processing chain has to be assessed. The aim of the present study was to screen for the occurrence of ESBL-producing *Enterobacteriaceae* in swine and cattle at slaughter and to further characterize isolated strains.

MATERIALS AND METHODS

Sampling. Fecal swab samples were collected in October 2009 from 123 animals: 59 pigs (57 fattening pigs and 2 piglets) and 64 cattle (10 cows, 7 young cows, 38 calves, 3 bullocks, and 6 fattening bulls). Swabs of fecal matter were taken from animals at a large slaughterhouse after cutting the large intestine with sterile scissors after evisceration. Afterward the swabs were placed into

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sterile tubes, transported to the laboratory, and processed within 4 h of collection. At most two animals per farm were sampled.

Microbiological analysis. Each swab was first suspended in 0.3 ml of 0.9% buffered peptone saline (BPS) and then incubated for 24 h at 37°C in *Enterobacteriaceae* enrichment (EE) broth (BD, Franklin Lakes, NJ). The BPS suspension and the enriched fecal samples were inoculated onto Brilliance ESBL agar (Oxoid, Hampshire, UK) and incubated at 37°C for 24 h under aerobic conditions. All colonies were selected and subcultured onto triple sugar iron agar (BD) at 37°C for 24 h. After performing the oxidase test and assessing for lactose fermentation, nonfermenters were discarded, and oxidase-negative colonies were subjected to identification by API ID 32 E (bioMérieux, Marcy l'Etoile, France) or by sequencing of 16S rRNA and *rpoB* genes (15, 20).

Antimicrobial susceptibility testing and ESBL detection. All isolated strains were subjected to susceptibility testing against 14 antimicrobial agents by the disc diffusion method and evaluated according to Clinical and Laboratory Standards Institute (3) criteria. The antibiotics tested were amoxicillin–clavulanic acid, cefpodoxime, cephalothin, cefepime, cefoxitin, cefotaxime (CTX), cefuroxime, ceftazidime, ampicillin, ciprofloxacin, streptomycin, gentamicin, chloramphenicol, and tetracycline (Becton Dickinson, Heidelberg, Germany). The amoxicillin–clavulanic acid disc was placed between the cefpodoxime and the ceftazidime discs, and the synergy effect was documented. The strains that showed a synergy effect were then confirmed as ESBL-producers on Mueller-Hinton agar plates using E-test ESBL strips containing cefotaxime, cefepime, or ceftazidime alone and in combination with clavulanic acid (bioMérieux), respectively, according to the manufacturer's recommendations. This included reading the MICs from the strips and calculating the ratio of (MIC cephalosporin)/(MIC cephalosporin plus clavulanic acid).

Characterization of β -lactamases. Bacterial strains confirmed as ESBL producers were further analyzed by PCR. DNA was extracted by a standard heat lysis protocol. Then five specific primer sets were used to determine the presence of the *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes (21, 25).

Genotyping. Pulsed-field gel electrophoresis was performed by following the CDC PulseNet protocol (<http://www.cdc.gov/pulsenet/protocols.htm>) with minor modifications. Briefly, strains were grown on blood agar at 37°C overnight. Colonies from blood agar were resuspended in cell suspension buffer (optical density at 600 nm = 1). The bacterial cell suspension was mixed with 400 μ l of 1.4% Bio-Rad Agarose (Bio-Rad, Munich, Germany), and cells were lysed by proteinase K treatment overnight. After lysis the plugs were washed twice for 15 min in ultrapure water and four times for an hour in Tris-EDTA buffer. After washing with Tris-EDTA buffer, DNA agarose plugs were incubated overnight in the presence of *Xba*I (Roche, Mannheim, Germany) following the manufacturer's instructions. Restricted DNA in plug slices was separated in a 1% SeaKem Gold (BioConcept, Allschwil, Switzerland) agarose gel at 6 V/cm in 0.5 \times Tris-borate-EDTA buffer cooled to 14°C in a CHEF-DR III system (Bio-Rad). The pulse times were ramped from 5 to 50 s for 20 h at an angle of 120°. Gels were stained with ethidium bromide and visualized under UV light transillumination using a Gel Doc system (Bio-Rad) and analyzed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Salmonella Braenderup strain H9812 (ATCC BAA 664) was used as a size standard.

RESULTS AND DISCUSSION

The 21 ESBL-producing strains were isolated from 20 animals (9 pigs, 1 cow, and 10 calves). All of the strains showed a synergy effect with at least one of three E-test ESBL strips containing cefepime, cefotaxime, or ceftazidime, and they yielded factors >8 when MIC ratios were calculated, such as (MIC cephalosporin)/(MIC cephalosporin plus clavulanic acid). Identification of the isolates resulted in 20 cases in *E. coli* and one in *Citrobacter youngae* (Table 1). Based on the MIC to oxyimino cephalosporins, four different resistotypes could be described. Resistotypes 1, 1a, 2, and 3 were characterized by differing MICs to the three oxyimino cephalosporins or by varying synergy effects (for more details see footnotes in Table 1).

The β -lactamase genes of all ESBL-producing isolates were further characterized by PCR. Seventeen (85%) of the *E. coli* ESBL genes belonged to CTX-M group 1, and three (15%; one porcine, two bovine samples) belonged to CTX-M group 9. The *C. youngae* isolate belonged to CTX-M group 1. None of the 21 ESBL producers harbored *bla*_{SHV}, but all of the CTX-M group 9–positive isolates and 29.4% of the CTX-M group 1–positive *E. coli* also harbored *bla*_{TEM}. The *C. youngae* isolate was negative for both *bla*_{TEM} and *bla*_{SHV}. In addition to these β -lactam resistances, we also tested for other classes of antibiotics; we found 15 strains resistant to tetracycline, 15 strains resistant to streptomycin, 10 strains resistant to chloramphenicol, 9 strains resistant to gentamicin, and 9 strains resistant to ciprofloxacin (Table 1).

There have only been a few studies describing the prevalence and characteristics of ESBL-producing *Enterobacteriaceae* in healthy farm animals. Very low prevalences were described for pigs in Denmark and cattle in France (17, 26). No prevalence data were available for Switzerland and other European countries. The predominance of CTX-M group 1 enzymes, as seen in our study, has also recently been described in strains from healthy food-producing animals in Denmark, Portugal, and France (1, 9, 10). In our study, 15% of the *E. coli* isolates belonged to CTX-M group 9. Notably, almost 30% of the ESBL-producing strains in the present study contained an additional *bla*_{TEM} gene. TEM producers are often isolated from poultry (22) but rarely from other animals (2, 4, 16). Although the *bla*_{TEM} genes were not further characterized, they can be expected to belong to the classical group of broad-spectrum β -lactamases conveying no ESBL phenotype.

The ESBL-producing strains showed high genetic diversity by pulsed-field gel electrophoresis analysis (Fig. 1). This is a matter of concern, since it shows that no single clone has spread within the animal population. Instead, propagation of resistance among *E. coli* populations in animals occurs more probably by plasmids harboring *bla*_{ESBL} genes.

The presence of ESBL-producing *Enterobacteriaceae* in fecal samples of farm animals represents a risk for carcass contamination at slaughter and therefore also indicates the potential for contamination of retail meat products. This, in turn, may lead to colonization of healthy humans with

TABLE 1. Identification and characterization of the 21 ESBL producers isolated from fecal samples of 59 pigs and 64 cattle at slaughter

| Sample no. | Animal | Identification ^a | Resistotype ^b | CTX-M group 1 | CTX-M group 2 | CTX-M group 9 | <i>bla</i> _{SHV} | <i>bla</i> _{TEM} | Other antibiotic resistances ^c |
|------------|--------|-----------------------------|--------------------------|---------------|----------------|---------------|---------------------------|---------------------------|---|
| 13 | Pig | <i>E. coli</i> | 1 | + | — ^e | — | — | — | S, TE |
| 14 | Pig | <i>E. coli</i> | 1 | + | — | — | — | — | TE |
| 16 | Pig | <i>E. coli</i> | 1a | — | — | + | — | + | S, C, TE, GM, CIP |
| 17 | Pig | <i>E. coli</i> | 1a | + | — | — | — | + | S, TE, CIP |
| 18 | Pig | <i>E. coli</i> | 1 | + | — | — | — | + | S, C, TE, CIP |
| 46 | Calf | <i>E. coli</i> | 2 | + | — | — | — | — | S, C, TE, GM |
| 47 | Calf | <i>E. coli</i> | 2 | + | — | — | — | — | S, C, TE, GM |
| 52 | Calf | <i>E. coli</i> | 3 | + | — | — | — | — | TE, GM, CIP |
| 53 | Cow | <i>E. coli</i> | 3 | + | — | — | — | — | CIP |
| 60 | Pig | <i>E. coli</i> | 1 | + | — | — | — | + | S |
| 64 | Pig | <i>E. coli</i> | 2 | + | — | — | — | — | — |
| 65 | Pig | <i>E. coli</i> | 2 | + | — | — | — | — | TE |
| 72 | Pig | <i>E. coli</i> | 1a | + | — | — | — | + | S |
| 112 | Calf | <i>E. coli</i> | 2 | + | — | — | — | — | S, C, TE, GM |
| 114 | Calf | <i>E. coli</i> | 2 | + | — | — | — | — | S, C, TE, GM, CIP |
| 115 | Calf | <i>E. coli</i> | 1a | — | — | + | — | + | C, CIP |
| 116 | Calf | <i>E. coli</i> | 1a | — | — | + | — | + | S, C, TE, GM, CIP |
| 124 | Calf | <i>E. coli</i> | 3 | + | — | — | — | — | S, CIP |
| 129 | Calf | <i>E. coli</i> | 2 | + | — | — | — | + | S, TE |
| 142a | Calf | <i>E. coli</i> | 2 | + | — | — | — | — | S, C, TE, GM |
| 142b | Calf | <i>C. youngae</i> | 2 | + | — | — | — | — | S, C, TE, GM |

^a *E. coli* identification by API ID 32; *C. youngae* identification by sequencing of 16S rRNA and *rpoB* genes.

^b Resistotype 1 (1 < MIC cefepime ≤ 8, synergy effect; MIC cefotaxime > 16, synergy effect; 0.5 ≤ MIC ceftazidime ≤ 1, synergy effect); resistotype 1a (like resistotype 1, but without synergy effect with strip cefotaxime/cefotaxime plus clavulanic acid); resistotype 2 (0.5 ≤ MIC cefepime ≤ 1, MIC cefotaxime ≥ 4, MIC ceftazidime ≤ 0.5); resistotype 3 (MIC cefepime ≥ 3, MIC cefotaxime > 16, MIC ceftazidime ≥ 4).

^c S, streptomycin; TE, tetracycline; C, chloramphenicol; GM, gentamicin; CIP, ciprofloxacin.

^d +, present.

^e —, absent.

ESBL-producing bacteria and thus may jeopardize subsequent antimicrobial therapies on carriers. Therefore, further efforts within the field of veterinary public health are of major importance. It is recommended that a monitoring

system be established to analyze further trends of ESBL frequencies, in order to prevent further corruption of the efficacy of the important oxyimino cephalosporins in the treatment of severe human infections.

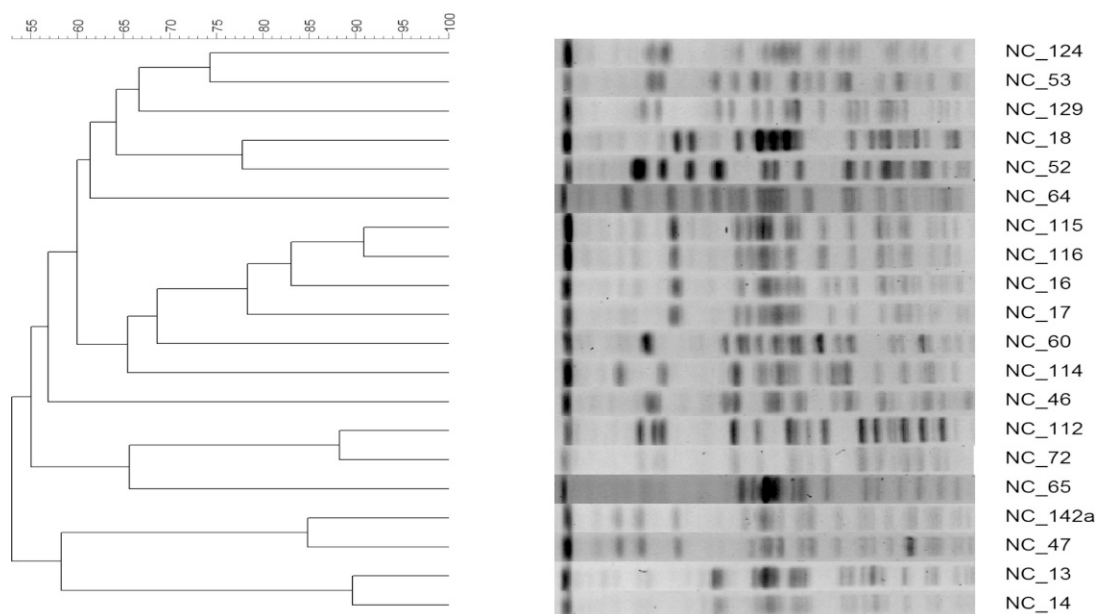


FIGURE 1. Pulsed-field gel electrophoresis dendrogram (Dice similarity index and unweighted pair group method with arithmetic mean) of the 20 ESBL-producing *E. coli* isolated from fecal samples of pigs and cattle at slaughter.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Veterinary Service at the Zurich slaughterhouse through giving access to the computer system to get the essential animal data about the samples collected. We thank the Federal Office of Public Health for partly funding the project.

REFERENCES

1. Aarestrup, F. M., H. Hasman, Y. Agerso, L. B. Jensen, S. Harksen, and B. Svensmark. 2006. First description of blaCTX-M-1-carrying *Escherichia coli* isolates in Danish primary food production. *J. Antimicrob. Chemother.* 57:1258–1259.
2. Blanc, V., R. Mesa, and M. Saco. 2006. ESBL- and plasmidic class C β -lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet. Microbiol.* 118:299–304.
3. Clinical and Laboratory Standards Institute. 2008. M100-S18. Performance standards for antimicrobial susceptibility testing, 18th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
4. Cloeckaert, A., K. Praud, B. Doublet, A. Bertini, A. Carattoli, P. Butaye, H. Imberechts, S. Bertrand, J.-M. Collard, G. Arlet, and F.-X. Weill. 2007. Dissemination of an extended-spectrum- β -lactamase bla_{TEM-52} gene-carrying IncII plasmid in various *Salmonella enterica* serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. *Antimicrob. Agents Chemother.* 51:1872–1875.
5. Coque, T. M., F. Baquero, and R. Canton. 2008. Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe. *Euro Surveill.* 13:19044.
6. Cortés, P., V. Blanc, A. Mora, G. Dahbi, J. E. Blanco, M. Blanco, C. López, A. Andreu, F. Navarro, M. P. Alonso, G. Bou, J. Blanco, and M. Llagostera. 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl. Environ. Microbiol.* 76:2799–2805.
7. Doi, Y., D. L. Paterson, P. Egea, A. Pascual, L. Lopez-Cerero, M. D. Navarro, J. M. Adams-Haduch, Z. H. Qureshi, H. E. Sidjabat, and J. Rodriguez-Bano. 2010. Extended-spectrum and CMY-type β -lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. *Clin. Microbiol. Infect.* 16:33–38.
8. Duan, R. S., T. H. Sit, S. S. Wong, R. C. Wong, K. H. Chow, G. C. Mak, W. C. Yam, L. T. Ng, K. Y. Yuen, and P. L. Ho. 2006. *Escherichia coli* producing CTX-M β -lactamases in food animals in Hong Kong. *Microb. Drug Resist.* 12:145–148.
9. Girlich, D., L. Poirel, A. Carattoli, I. Kempf, M. F. Lartigue, A. Bertini, and P. Nordmann. 2007. Extended-spectrum (beta)-lactamase CTX-M-1 in *Escherichia coli* in healthy poultry in France. *Appl. Environ. Microbiol.* 73:4681–4685.
10. Gonçalves, A., C. Torres, N. Silva, C. Carneiro, H. Radhouani, C. Coelho, C. Araújo, J. Rodrigues, L. Vinué, S. Somalo, P. Poeta, and G. Igrejas. 2010. Genetic characterization of extended-spectrum β -lactamases in *Escherichia coli* isolates of pigs from a Portuguese intensive swine farm. *Foodborne Pathog. Dis.* 7:1569–1573.
11. Hammad, A. M., A. M. Ahmed, Y. Ishida, and T. Shimamoto. 2008. First characterization and emergence of SHV-60 in raw milk of a healthy cow in Japan. *J. Vet. Med. Sci.* 70:1269–1272.
12. Jensen, L. B., H. Hasman, Y. Agerso, H. D. Emborg, and F. M. Aarestrup. 2006. First description of an oxyimino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. *J. Antimicrob. Chemother.* 57:793–794.
13. Jouini, A., L. Vinué, K. B. Slama, Y. Sáenz, N. Klibi, S. Hammami, A. Boudabous, and C. Torres. 2007. Characterization of CTX-M and SHV extended-spectrum β -lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J. Antimicrob. Chemother.* 60:1137–1141.
14. Knothe, H., P. Shah, V. Krcmery, M. Antal, and S. Mitsuhahi. 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 11:315–317.
15. Lehner, A., T. Tasara, and R. Stephan. 2004. 16S rRNA gene based analysis of *Enterobacter sakazakii* strains from different sources and development of a PCR assay for identification. *BMC Microbiol.* 4:43.
16. Machado, E., T. M. Coque, R. Canton, J. C. Sousa, and L. Peixe. 2008. Antibiotic resistance integrons and extended-spectrum β -lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal. *J. Antimicrob. Chemother.* 62:296–302.
17. Madec, J. Y., C. Lazizzera, P. Châtre, S. Martin, G. Lepage, M. F. Ménard, P. Lebreton, T. Rambaud, and D. Meunier. 2008. Prevalence of fecal carriage of acquired expanded-spectrum cephalosporin resistance in *Enterobacteriaceae* strains from cattle in France. *J. Clin. Microbiol.* 46:1566–1567.
18. Mesa, R. J., V. Blanc, A. R. Blanch, P. Cortés, J. J. González, S. Lavilla, E. Miró, M. Muniesa, M. Saco, M. T. Tórtola, B. Mirelis, P. Coll, M. Llagostera, G. Prats, and F. J. Navarro. 2006. Extended-spectrum β -lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). *J. Antimicrob. Chemother.* 58:211–215.
19. Meunier, D., E. Jouy, C. Lazizzera, M. Kobisc, and J. Y. Madec. 2006. CTX-M-1- and CTX-M-15-type β -lactamases in clinical *Escherichia coli* isolates recovered from food-producing animals in France. *Int. J. Antimicrob. Agents* 28:402–407.
20. Mollet, C., M. Drancourt, and D. Raoult. 1997. *rpoB* sequence analysis as a novel basis for bacterial identification. *Mol. Microbiol.* 26:1005–1011.
21. Pitout, J. D., K. S. Thomson, N. D. Hanson, A. F. Erhardt, E. S. Moland, and C. C. Sanders. 1998. Beta-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. *Antimicrob. Agents Chemother.* 42:1350–1354.
22. Smet, A., A. Martel, D. Persoons, J. Dewulf, M. Heyndrickx, B. Catry, L. Herman, F. Haesebrouck, and P. Butaye. 2008. Diversity of extended-spectrum β -lactamases and class C β -lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. *Antimicrob. Agents Chemother.* 52:1238–1243.
23. Tian, G. B., H. N. Wang, L. K. Zou, J. N. Tang, Y. W. Zhao, M. Y. Ye, J. Y. Tang, Y. Zhang, A. Y. Zhang, X. Yang, C. W. Xu, and Y. J. Fu. 2009. Detection of CTX-M-15, CTX-M-22, and SHV-2 extended-spectrum β -lactamases (ESBLs) in *Escherichia coli* fecal-sample isolates from pig farms in China. *Foodborne Pathog. Dis.* 6:297–304.
24. Wittum, T. E., D. F. Mollenkopf, J. B. Daniels, A. E. Parkinson, J. L. Mathews, P. R. Fry, M. J. Abley, and W. A. Gebreyes. 2010. CTX-M-type extended-spectrum β -lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. *Foodborne Pathog. Dis.* 7:1575–1579.
25. Woodford, N., E. J. Fagan, and M. J. Ellington. 2005. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β -lactamases. *J. Antimicrob. Chemother.* 57:154–155.
26. Wu, S., E. Chouliara, H. Hasman, A. Dalsgaard, A. Vieira, and L. B. Jensen. 2008. Detection of a single isolate of CTX-M-1-producing *Escherichia coli* from healthy pigs in Denmark. *J. Antimicrob. Chemother.* 61:747–749.